NON-CANCER ENDPOINTS

There are perhaps three considerations that distinguish the health risk evaluation process for cancer endpoints from that for non-cancer endpoints.

I. While carcinogenic effects are thought to be linear with dose all the way to zero dose, for non-cancer endpoints there exists a threshold dose level below which no adverse health effects occur. This level is typically called the reference dose (RfD), allowable intake chronic (AIC), or no observed adverse effect level (NOAEL).

II. The contrast between target tissues and the rest of the body is generally more sharply drawn than in carcinogenesis. That is, with non-cancer endpoints the target tissue/organ is often exquisitely susceptible to harm in comparison to other body tissues. Calculation of health effects often calls for the use of physiologically-based pharmacokinetics (PB-PK), so that dose to target tissues can be more closely estimated.

III. Non-cancer endpoints of injury are much more widely varied and toxin-specific than in cancer, where we believe there is primarily one endpoint, genetic damage, and one outcome, death, that we seek to avoid.

Because of the diversity in non-cancer endpoints, it would be impossible to present an overall survey, and one example will be discussed in some depth. Many of the principles can be extrapolated to other organ systems.

Assessment of Risk for Inhaled Airborne Material

There are many methods available to assess the toxicity of inhaled agents. As summarized below, these tests range from studies in human populations, to measures of lung function in whole animals and histopathological studies of lungs from exposed animals, to *in vitro* measures of pulmonary macrophage function (phagocytosis, viability), etc. The following outline describes various categories of lung injury and types of assays for indicating onset of tissue damage.

I. Inhalation toxicology data development

A. Air monitoring and characterization of collected dusts.

B. Epidemiologic studies of previously-exposed populations.

C. Clinical trials using controlled exposures of humans.

D. Animals, chronic lifetime studies.

E. Short term animal bioassays.

F. *In vitro* tests on mammalian or non-mammalian cells.

G. *In vitro* examination of molecular interactions with phospholipids, enzymes, nucleic acids, etc.

II. Mechanisms of lung injury

As a consequence of inhaling toxic gases and particles, a number of pathological processes may be set into motion. None are specific to the lung, but their expression and consequences depend on the unique architecture and physiological role of the respiratory system. Major pathological mechanisms to be discussed are:

A. Pulmonary edema: Transudation of fluid, altered alveolar stability, impaired gas exchange, and respiratory distress

B. Inflammation: Irritation leading to mucosal edema, increased mucus production and bronchitis, appearance of neutrophils and inflammatory mediators, increased cell renewal

C. Immunologic reactions: Asthma, hypersensitivity lung disease, extrinsic allergic alveolitis, anaphylaxis

D. Altered susceptibility to infection: Cytotoxic and competitive effects on macrophage function, altered mucociliary transport because of changes in cilia or the quantity or rheological character of mucus

E. Infection: Bacterial, viral, or fungal pneumonia

F. Proteolysis: Destruction of elastin and collagen leading to emphysema, obstructive lung disease

G. Fibrosis: Increased connective tissue scarring, excessive collagen, restrictive lung disease

H. Degenerative changes: Necrosis, calcification, and autolysis

I. "Pulmonary carcinogenesis: bronchogenic carcinoma, oat cell carcinoma, adenocarcinoma, mesothelioma"

III. Measurement of lung injury

If the lung is injured by inhaled toxic gases and particles, how can the lung injury be detected and quantified? What repertoire of approaches can be used?

Approaches and Parameters or Methods:

A. Mechanical properties (pulmonary function)

1. Resistance
2. Compliance: pressure-volume curves
3. Lung volumes: VC (spirometry), TLC, RV, and FRC (measured by helium dilution, Boyle's law)
4. $FEV_{1.0}$ and Full or Partial flow-volume curves

B. Gas exchange, Adequacy of ventilation, Distribution of ventilation and perfusion

1. Alveolar gas tensions (P_ACO_2, P_AO_2)
2. Arterial p_aCO_2, p_aO_2
3. Ventilation homogeneity: N_2 washout
4. Ventilation (^{133}Xe) or Perfusion (^{67}Ga) scans

5. a-A concentration gradients
6. Diffusing capacity (carbon monoxide uptake)

C. Measurement of pathology by radiologic techniques

1. Atelectasis
2. Fibrosis, emphysema, etc.
3. Bronchography (Tantalum)
4. Focal lesions

D. Mucociliary transport (*in vitro* and *in vivo*)

1. Nasal
2. Airways
3. Mucus studies
4. Cilia studies

E. Lung lavage parameters

1. Surfactant: quantity, composition
2. Cell numbers, appearance, and viability
3. Cell differential counts: RBC's, PMN's, monocytes, macrophages, lymphocytes
4. Proliferation: production of colony-forming units (CFU's) by lavaged cells, uptake of tritiated thymidine
5. Mucus constituents
6. Biochemistry: albumin, hemoglobin, hydroxyproline, elastase, collagenase, LDH, myeloperoxidase, antiproteases, lysosomal enzymes, active oxygen species, chemotaxins, proliferative factors, and inflammatory mediators (histamine, prostaglandins, leukotrienes)
7. *In vitro* functional assays of macrophage activity: trypan blue dye exclusion, oxygen consumption, ATP levels, lactate production, migration, chemotactic responsiveness, phagocytosis, killing of microorganisms, release of mediators

F. Morphology

1. Gough sections
2. Reid index
3. Morphometric approaches: airway and alveolar dimensions
4. Cell types: connective tissue, inflammatory, neoplastic
5. Proliferation and cell turnover measures
6. Vascular changes

G. Renewal of lung constituents observed in tissue sections

1. Metaphase counts - colchicine
2. Uptake of tritiated thymidine
3. Collagen and elastin breakdown and synthesis

H. Lung clearance

1. DTPA-measured lung epithelial permeability
2. Clearance of radioactively-labelled inhaled particles
3. Clearance of magnetic inhaled particles
4. Macrophage motile activity measured by inhaled magnetic particles

I. Microbicidal activity

1. Recognizable experimental pulmonary infections (morbidity and mortality studies)
2. Bacterial aerosol models, *in vivo* models
3. *In vitro* killing
4. Phagocytosis: *in vitro* and *in vivo*

J. Identifying pulmonary carcinogens

1. Experimental pulmonary carcinogenesis (Saffiotti model)
2. Chromosome abnormalities
3. Ames mutagenesis assay

IV. Bioassays for measuring toxicity of particles and components of particles

A. Whole animals

B. *In vitro* cell culture systems

C. Cell homogenates

V. Questions to be considered in the interpretation of data

A. Species extrapolation. Are human and animal toxicities equivalent ?

B. Dose extrapolation. Are the doses given to animals comparable to human exposures ?

C. Time extrapolation. At what stage is the injury being measured, and how does it compare to the time course of disease development in humans ?

D. Correlation of disease mechanism with bioassay result

E. Specificity of bioassay result: Is result unique to the agent tested ? Is the result generalizable to a class of agents ? If the agent is a complex mixture, what are the active components ? How does the bioassay result agree with disease outcomes in cases where human data are available ?

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Figure 3